

1 REMARKS

2 Status of the Claims

3 Claims 1-24, 29-31, 33, 34, 36, 37, 39, 40, and 45-48 are now pending in the present application,  
4 Claims 25 and 26 having been previously canceled, and Claims 27, 28, 32, 35, 38, and 41-44 having been  
5 canceled herein, and Claims 45-48 having been added herein. Claims 16, 23, 24, 29, 30, 31, 33, 34, 36,  
6 37, 39 and 40 have been amended to more clearly define the subject matter being claimed.

7 Definition of Spatial Frequency Content

8 The pending claims each recite the use of a *spatial frequency content* metric that does not appear  
9 to be employed by prior art cytometry based cell classifiers. For example, Claim 1 specifically recites  
10 using a particular morphological parameter defined from a side scatter image of a cell that applicants have  
11 determined is very important in providing a simple and effective cell classifier. That parameter is *spatial*  
12 *frequency content data from a side scatter image* (a side scatter image is also referred to as a  
13 darkfield image).

14 In non-imaging flow cytometers, laser light is used to illuminate a cell, and a photodetector is  
15 used to collect amplitude data (i.e., intensity data) corresponding to light received from the cell. Such  
16 data often includes forward scatter (FSC) intensity data and side scatter (SSC) intensity data. The  
17 Nicoletti and Vitale references (the Purdue references) discuss uses of FSC data and SSC data.  
18 Significantly, the FSC and SSC data is collected by non-pixelated, non-imaging detectors. The FSC and  
19 SSC data is simply an amplitude or intensity measurement about the total quantity of light received at the  
20 detector.

21 It is important to recognize the inherent limitation of intensity measurements from non-imaging  
22 cytometers. In the prior art, intensity data of SSC from non-imaging cytometers was known to provide  
23 *some* information about the texture or internal complexity of a cell, while intensity data of FSC light from  
24 non-imaging cytometers was known to provide *some* information about the relative size of a cell (Vitale,  
25 page 4, Background Information). However, these measurements were qualitative in nature, and were  
26 generally insufficient for conclusive classification of a cell. For example, a relatively small cell having  
27 few light absorbing internal structures could provide the same FSC intensity measurement as a relatively  
28 larger size cell having more light absorbing internal structures. Thus, the FSC intensity measurement did  
29 not really provide an accurate measurement of the size of a cell, and cell classification based solely on  
30 FSC and SSC measurements was error prone.

1 In the context of the present application, it must be recognized that the data being collected from a  
2 cell is not a single numerical value corresponding to the intensity of FSC light collected from a cell, or  
3 SSC light collected from a cell. Rather, pixilated data (i.e., an image) of the cell is collected in multiple  
4 channels, and includes several fluorescent images of the cell, a forward scatter image of the cell, and a  
5 side scatter image of the cell. In particular, the term *spatial frequency content data from a side scatter*  
6 *image* refers to a parameter that can only be acquired from an image (i.e., from a pixilated detector).  
7 The photo detectors used in non-imaging cytometers are not pixilated detectors, and only acquire an  
8 amplitude signal, not an image.

9 The term *spatial frequency* or *spatial frequency content* has a recognized meaning in the  
10 signal processing arts. Applicants did not specifically define the term in the application as filed,  
11 simply because its accepted meaning is so well known in the signal processing arts that there  
12 appeared no need to define the term. For example, Wikipedia defines spatial frequency as follows  
13 (noting that applicants are confident that any signal processing reference will provide a similar  
14 definition): *In mathematics, physics, and engineering, **spatial frequency** is a characteristic of any*  
15 *structure that is periodic across position in space. The spatial frequency is a measure of how often*  
16 *sinusoidal components (as determined by the Fourier transform) of the structure repeat per unit of*  
17 *distance. The SI unit of spatial frequency is cycles per meter. In image processing applications,*  
18 *spatial frequency is often expressed in units of cycles per millimeter and sometimes incorrectly in*  
19 *units of line pairs per millimeter (incorrect since a line pair may describe a square wave but not a*  
20 *sinusoidal wave).*

21 Thus, applicants can reasonably expect the artisan of skill in image processing to recognize  
22 that the *spatial frequency content data* from an image of a cell is a measurement of how pixels in an  
23 image vary from other pixels in that image (i.e., a measurement of the variation or periodicity of  
24 pixels in the image, each pixel being separated from other pixels by a certain distance).

25 MPEP 2111.01 specifically requires claim terms to be given their plain meaning, based on the  
26 ordinary and customary meaning the term would have to a person of ordinary skill in the art in  
27 question. Clearly, the term *spatial frequency content data from a side scatter image* must be  
28 interpreted consistent with how one of ordinary skill in the image processing arts would understand  
29 the term. MPEP 2111.01 further suggests that where the term is a *technical term*, consultation of  
30 extrinsic sources, such as dictionaries, can be used to define the term appropriately. Some terms have

1 multiple dictionary definitions, and in that case care must be taken to ensure the appropriate  
2 definition is employed. In context of the present application, applicants know of no other accepted  
3 definition of the *spatial frequency content of an image* than the one provided above (i.e., a  
4 measurement of how often image components (as determined by the Fourier transform) of the  
5 structure repeat per unit of distance). Unless the Examiner is aware of other extrinsic sources that  
6 provide a different definition, or is aware of some reason that an artisan of skill in the image  
7 processing arts would interpret the term differently, it appears that applicants are entitled to have the  
8 term interpreted as noted above.

9 The reason applicants consider this an issue is because some of the cited non-imaging  
10 cytometry references refer to SSC light intensity measurements, which are not equivalent to the  
11 claimed *spatial frequency content* metric. A SSC light measurement in non-imaging flow cytometry  
12 is a simple amplitude or intensity measurement of side scattered light. The SSC light intensity  
13 measurement does not measure how image components repeat per unit of distance. Simply because  
14 both types of data are generated using side scattered light does not mean they are equivalent. Any  
15 rejection of Claim 1 must take those differences into account. Thus, any rejection of Claim 1 should  
16 be based on a reference that discloses using *spatial frequency content data from a side scatter image*  
17 in an equivalent manner, or which discloses that *spatial frequency content data from a side scatter*  
18 *image* can be used as an equivalent for an intensity measurement of side scattered light from a cell.

19 Should the Examiner intend to interpret *spatial frequency content data from a side scatter*  
20 *image* (or brightfield image, or other image of the cell) in a manner inconsistent with the above  
21 discussion, applicants respectfully request that the Examiner enter into the record an articulation of  
22 the reasoning behind concluding that a different interpretation is merited, particularly in light of  
23 MPEP 2111.01.

24 In some dependent claims, applicants have further defined the term spatial frequency content,  
25 based on disclosure in paragraph [0045] of the specification as filed, which states: *The 488 nm*  
26 *spatial scatter frequency can be calculated by computing the standard deviation of the individual*  
27 *pixel intensities within the segmented dark field image mask.* However, applicants do believe that the  
28 term spatial frequency content (or simply spatial frequency) is sufficiently well understood in the  
29 image processing arts that no additional definition is needed to distinguish over FSC intensity  
30 measurements and SSC intensity measurements in non-imaging (i.e.; conventional) cytometry.

1 Discussion of the Closest Prior Art and Significant Elements of Applicants' Claims

2 Before addressing the Office Action in detail, applicants believe it may be helpful to discuss what  
3 appear to be the most closely related art, the Vitale and Nicolletti references (the Purdue cytometry  
4 references). Each reference discusses non-imaging flow cytometry, and how such non-imaging flow  
5 cytometry can be used to classify cells as necrotic, viable, early apoptotic, or later apoptotic.  
6 Significantly, applicants believe that while these references are somewhat successful in classifying cells in  
7 the four categories identified above, neither of these references teach or suggest a single technique that  
8 can accurately classify cells as belonging to one of those four categories, using only a single metric  
9 (spatial frequency content), and in some of the pending claims, a single nuclear stain, and without  
10 requiring some additional technique for verification of the results (note that the Vitale and Nicolletti  
11 references appear to suggest that the non-imaging flow cytometry techniques are error prone and that their  
12 results should be validated using manual microscopy).

13 Nicoletti (*Common Methods for Measuring Apoptotic Cell Death by Flow Cytometry*) appears to  
14 disclose a first technique (see pages 3-6) that uses a single nuclear stain to distinguish between late state  
15 apoptotic cells, necrotic cells, and viable cells. The viable cells (and early stage apoptotic cells) have  
16 intact membranes that reject the nuclear stain (thus are negative for the stain). The late stage apoptotic  
17 cells stain to a lesser degree than necrotic cells. Thus, a population of cells can be separated into the  
18 following three groups based on the intensity of the stain signal: viable (and early apoptotic), late stage  
19 apoptotic, and necrotic. Note this technique **cannot** differentiate the viable and early apoptotic cells,  
20 because both cell types prevent the stain from crossing the cellular membrane. Further, at page 6,  
21 Nicoletti expressly teaches that direct microscopic evaluation of cell suspensions is necessary to obtain  
22 reproducible results.

23 Nicoletti also discloses a different technique that can be used to identify early apoptotic cells from  
24 other cell types. This technique is based on staining DNA breaks (early apoptotic cells have more DNA  
25 breaks than other cell types, thus a relatively high fluorescent signal from the break dye is indicative of  
26 early apoptotic cells, while a relatively lower dye signal is indicative of viable or necrotic cells). Another  
27 technique uses a stain that selectively binds to a protein expressed during the early stage of apoptosis  
28 (Annexin-V; see page 8). Significantly, Nicoletti summarizes his findings by explicitly stating that *no*  
29 *single method can provide unequivocal quantitative measure of apoptosis in all situations* (see page 9).

1 Applicants respectfully submit that the pending claims do encompass such a technique, and represent a  
2 non-obvious modification of Nicoletti's disclosure.

3 One of the reasons that Nicoletti's cannot achieve a single analysis that can distinguish all four  
4 cell types using a single stain is that the SSC and FSC intensity measurements simply do not provide  
5 sufficient quantitative data. The spatial frequency content of image data provides a much more detailed  
6 data set, in that not only is the intensity of each pixel in the image measured, the spatial frequency metric  
7 provides a measure of how each pixel varies from other pixels in the image. This provides significantly  
8 more data, which applicants have learned to exploit to achieve a single analysis that can differentiate each  
9 of the four cell types (early apoptotic, late apoptotic, viable, and necrotic), a technique Nicoletti failed to  
10 achieve.

11 Referring now to Vitale (Apoptosis versus Necrosis), this reference discloses using a combination  
12 of FSC intensity measurements and SSC intensity measurements to classify cells as necrotic, late  
13 apoptotic, and viable. Significantly, one of the first statements in Vitale's disclosure (item 1, page 2) is  
14 that *A univocal method to distinguish apoptotic from necrotic cells by flow cytometry does not exist.*  
15 *Rather it is necessary to discriminate necrotic cells from apoptotic cells in each of many flow cytometric*  
16 *methodologies available to measure apoptosis.*

17 It must be emphasized that applicants disclose and claim such a method (i.e., a single analytical  
18 technique, that using single nuclear stain, can classify cells as either necrotic, late apoptotic, early  
19 apoptotic, or viable). Vitale clearly recognizes that his disclosed technique has not achieved that single  
20 technique.

21 Vitale does disclose that viable cells will have mid range SSC values and mid-high range FSC  
22 values (bottom of page 3), that necrotic cells will have lower SSC values and lower FSC values (top of  
23 page 4), and that apoptotic cells will have higher SSC values and lower FSC values (top of page 4).  
24 Significantly, this technique does not appear to encompass early stage apoptotic cells (only in late stage  
25 apoptosis is the cytoplasm and plasma membrane seriously damaged; bottom of page 4).

26 A latter section of Vitale discussing a nuclear staining technique (the SSC and FSC technique  
27 above is not based on staining) which employs *two* nuclear stains to facilitate the classification of cells as  
28 necrotic or apoptotic (bottom of page 7). Apoptotic cells show high HO staining and low PI staining,  
29 necrotic cells are brightly stained by PI, and healthy cells are dimly stained by HO and not stained by PI.

1 It also must be recognized that Vitale clearly suggests that after flow cytometry analysis,  
2 morphological analysis must be used to confirm the data (item 4, page 3). Significantly, the  
3 morphological analyses disclosed by Vitale are not cytometry based, but microscopy based (electron  
4 microscopy, both SEM and TEM (page 9 and beyond)).

5 Clearly, Vitale discloses cytometry based intensity measurement techniques (SSC and FSC) that  
6 can differentiate between late apoptotic, viable, and necrotic cells, and dual staining techniques that can  
7 distinguish between late apoptotic, viable, and necrotic cells, both of which should be validated using  
8 electron microscopy (a slow, tedious technique). Vitale does not teach or suggest analyzing the spatial  
9 frequency of an image of a cell, and using that parameter as a cell classifier, nor a single cell classification  
10 technique that can differentiate between early apoptotic, late apoptotic, viable, and necrotic cells using a  
11 single nuclear stain and a single analysis.

12 37 C.F.R. 1.132 Declaration Attributing U.S. Patent Publication No. 2004/0021868 to Applicants

13 Applicants respectfully submit that U.S. Patent Publication No. 2004/0021868 is not prior art,  
14 because the reference is applicants' own disclosure published less than 1 year before the filing date of the  
15 provisional application (60/553,502) for which the pending application claims priority.

16 The provisional application was filed on March 16, 2004.

17 The pending application includes the following inventors. Thaddeus George, David Basiji, Brian  
18 Hall, William Orтын, Michael Seo, Philip Morrissey, and Cathleen Zimmerman.

19 U.S. Patent Publication No. 2004/0021868 was published on February 5, 2004. The inventors of  
20 U.S. Patent Publication No. 2004/0021868 are David Basiji and William Orтын.

21 Various portions of MPEP 715 discuss fact patterns where there is some overlap in  
22 inventorship between a reference and a pending application, but none of the those fact patterns match  
23 the present fact pattern (where inventorship of the pending application is different than the  
24 inventorship/authorship of the reference, but EVERY author in the reference is an inventor in the  
25 pending application.)

26 715.01(a) notes that: *When subject matter, disclosed but not claimed in a patent or*  
27 *application publication filed jointly by S and another, is claimed in a later application filed by S, the*  
28 *joint patent or application publication is a valid reference under 35 U.S.C. 102(a) or (e) unless*  
29 *overcome by affidavit or declaration under 37 CFR 1.131 or an unequivocal declaration under*  
30 *37 CFR 1.132 by S that he/she conceived or invented the subject matter disclosed in the patent or*

1 *application publication and relied on in the rejection.* Note the affidavit is required because it is  
2 possible that a party other than one of the inventors in the pending application could have been  
3 responsible for the subject matter in question. In the present fact pattern, there simply is no  
4 possibility that the subject matter in the reference could be attributed to anyone other than David  
5 Basiji or William Ortyn, who are also listed inventors in the pending application. Logically, even  
6 though the inventive entities in the reference and the pending application are not identical, because  
7 every inventor in the reference is an inventor in the pending application, no affidavit should be  
8 necessary to establish that the common subject matter was not developed by a party other than a  
9 named inventor in the pending application.

10 However, in the interest of advancing prosecution of the present application, applicants have  
11 submitted herewith declarations from David Basiji and William Ortyn that the subject matter  
12 disclosed in U.S. Patent Publication No. 2004/0021868 is their own, and that they are co-inventors in the  
13 pending application.

14 Drawing Objection

15 Claim 43 has been canceled, rendering the objection moot.

16 Claims Rejected under 35 U.S.C. 112, Second Paragraph

17 The Examiner has rejected Claims 30-44 as being indefinite for the use a subjective term that is  
18 not defined in the specification (the term “relatively”). The claims have been amended to cancel the term,  
19 rendering the rejection moot.

20 Claims Rejected under 35 U.S.C. 102(b)

21 The Examiner has rejected Claims 27 and 28 under 35 U.S.C. § 102(b) as being anticipated by  
22 U.S. Patent Publication No. 2002/0159625 (Elling). Applicants have canceled Claims 27 and 28,  
23 rendering the rejection moot.

24 Claims Rejected under 35 U.S.C. 102(e)

25 The Examiner has rejected Claim 29 under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent  
26 Publication No. 2004/0021868 (Ortyn). The above noted declaration should clarify that U.S. Patent  
27 Publication No. 2004/0021868 refers to a publication (whose publication is less than 1 year from the  
28 pending application’s priority date) solely attributable to named inventors of the pending application, and  
29 that U.S. Patent Publication No. 2004/0021868 is not prior art.

1 Claims Rejected under 35 U.S.C. § 103(a)

2        Claims 1-23 are rejected under 35 U.S.C. § 103(a) as unpatentable over U.S. Patent Publication  
3 No. 2004/0021868 (Ortyn) in view of a publication by Young et al. (*Towards automatic cell identification*  
4 *in DIC microscopy*). Young discloses using FFT analysis of DIC image data to match the sample data to  
5 templates, to identify individual yeast cells. The Examiner argues that it would have been obvious to use  
6 an automated classifier such as that disclosed by Young to analyze the data acquired by Ortyn's imaging  
7 device. The discussion above should clarify that U.S. Patent Publication No. 2004/0021868 refers to a  
8 publication (whose publication is less than 1 year from the pending application's priority date) solely  
9 attributable to named inventors of the pending application, and that U.S. Patent Publication No.  
10 2004/0021868 is not prior art. Thus, the obviousness rejection based on U.S. Patent Publication No.  
11 2004/0021868 is not valid.

12        DIC refers to Differential interference contrast microscopy (DIC), also known as Nomarski  
13 Interference Contrast (NIC) or Nomarski microscopy, which is an optical microscopy illumination  
14 technique used to enhance the contrast in unstained, transparent samples. DIC works on the principle  
15 of interferometry to gain information about the optical density of the sample, to see otherwise  
16 invisible features. A relatively complex lighting scheme produces an image with the object appearing  
17 black to white on a grey background. This image is similar to that obtained by phase contrast  
18 microscopy but without the bright diffraction halo. Young discloses that DIC images of known cells  
19 are used to define templates, and then DIC images of unknown cells are compared to the templates to  
20 determine if any of the templates correspond to the unknown cell. A fast Fourier transform (FFT)  
21 algorithm can be used to reduce the computational requirements in the comparison of the unknown  
22 DIC image to the templates.

23        Applicants recognize that the imaging system used to acquire the data that is used in the pending  
24 application to classify cells (as one of a viable cell, a necrotic cell, an early apoptotic cell or a late  
25 apoptotic cell) has been publicly disclosed more than a year before the filing date of the pending  
26 application (the Examiner has previously cited to earlier patents disclosing the imaging system). Those  
27 earlier publications specifically disclose a cellular biological related analysis that could be efficiently  
28 implemented by the imaging system (the FISH spot analysis). However, those earlier publications simply  
29 do not teach which of the many disclosed metrics (including nuclear area, perimeter, texture or spatial  
30



1 frequency content, centroid position, shape, volume, and ratios of such parameters) could be used to  
2 achieve a cell classifier to identify the viability of a cell.

3 Thus, even though Young discloses that a cell classifier based on DIC images and templates of  
4 known cells is known (noting that Young's cell classifier is not directed to defining the viability of the  
5 cell), the cited art does not teach or suggest using the specific metrics cited in the pending claims (i.e.,  
6 the spatial frequency content metric). For example, independent Claim 1 recites using the metric of a  
7 spatial frequency content of the *darkfield/side scatter image of a cell* (not the DIC image disclosed by  
8 Young), independent Claim 8 recites using the metric of a spatial frequency content of the *brightfield*  
9 *image of a cell* (not the DIC image disclosed by Young), and independent Claim 16 recites using the  
10 metric of a *spatial frequency content of an image of a cell stained with a nuclear stain* (Young's  
11 technique does not appear to be based on any nuclear stain). Young does not teach or suggest the use  
12 of the images specifically recited by applicants (darkfield or brightfield) as the basis for a cell classifier,  
13 or the spatial frequency metric recited by applicants.

14 The cited art does not teach or suggest a method for cell classification using metrics equivalent to  
15 those recited in independent Claims 1, 8 and 16. Because dependent claims inherently include each  
16 element recited in the independent claim upon which they ultimately depend, each claim depending  
17 upon independent Claims 1, 8 and 16 are patentable for at least the same reasons as those discussed  
18 above. Accordingly, the rejection of dependent Claims 2-7, 9-15, and 17-23 as being obvious in view  
19 of Young should be withdrawn.

#### 20 Claims Rejected under 35 U.S.C. § 103(a)

21 Claim 24 has been rejected under 35 U.S.C. § 103(a) as unpatentable over U.S. Patent Publication  
22 No. 2004/0021868 (Ortyn) in view of a publication by Young et al. (*Towards automatic cell identification*  
23 *in DIC microscopy*), further in view of U.S. Patent No. 5,372,936 (Fratz). Claim 24 specifically recites  
24 that the nuclear marker is 7-AAD.

25 As noted above, U.S. Patent Publication No. 2004/0021868 should not be considered to be prior  
26 art, thus the obviousness rejection based on U.S. Patent Publication No. 2004/0021868 is not valid.

27 With respect to Fratz disclosing 7-AAD as a nuclear marker, applicants recognize that 7-ADD is a  
28 known nuclear marker. What is significant about Claim 24 is that applicants identified a way to use  
29 image data acquired by the disclosed imaging system and *only one single nuclear marker* to achieve a cell  
30 classifier to determine if a given cell is a viable cell, a necrotic cell, an early apoptotic cell or a late

1 apoptotic cell. In other words, applicants believe that they are the first to develop a cell viability classifier  
2 that can determine if a given cell is a viable cell, a necrotic cell, an early apoptotic cell or a late apoptotic  
3 cell **using only a single nuclear marker.**

4 Claims Rejected under 35 U.S.C. § 103(a)

5 Claims 30-41 and 44 have been rejected under 35 U.S.C. § 103(a) as unpatentable over U.S.  
6 Patent Publication No. 2004/0021868 (Ortyn) in view of a publication by Nicoletti (*Common Methods for*  
7 *Measuring Apoptotic Cell Death in Flow Cytometry*), and further in view of U.S. Patent No. 5,372,936  
8 (Fratz), which discloses using a specific nuclear stain.

9 As noted above, U.S. Patent Publication No. 2004/0021868 should not be considered to be prior  
10 art, thus the obviousness rejection based on U.S. Patent Publication No. 2004/0021868 is not valid.  
11 Further, applicants note that independent Claim 29 and its dependent claims have been extensively  
12 amended. The remarks below briefly discuss how the amended claims distinguish over the cited art,  
13 including Nicoletti.

14 Claim 29 as amended (note Claim 30-41 and 44 depend upon Claim 29) recites using spatial  
15 frequency content data from an image of a cell stained with only a single nuclear marker to conclusively  
16 determine whether the cell is viable, early apoptotic (early being defined as having the cell membrane  
17 intact), late apoptotic (late being defined as having the cell membrane not intact), and necrotic. The cited  
18 art (specifically Vitale and Nicoletti) can use stains, FSC intensity data, and SSC intensity data to classify  
19 cells as in one or more of those groups, ***but not all of those groups by using only a single nuclear stain.***  
20 Further, the prior art does not use the spatial frequency content metric of a cellular image as part of a cell  
21 classifier.

22 The cited art does not teach or suggest a method for cell classification using metrics equivalent to  
23 those recited in independent Claim 29, using only a single nuclear stain. Because dependent claims  
24 inherently include each element recited in the independent claim upon which they ultimately depend,  
25 each claim depending upon independent Claim 29 is patentable for at least the same reasons as those  
26 discussed above. Accordingly, the rejection of dependent Claims 30-41 and 44 as being obvious in  
27 view of Nicoletti and Fratz should be withdrawn (noting that Claims 32, 35, 38, 41 and 44 have been  
28 canceled).

1 Claims Rejected under 35 U.S.C. § 103(a)

2 Claims 42 and 43 have been rejected under 35 U.S.C. § 103(a) as unpatentable over U.S. Patent  
3 Publication No. 2004/0021868 (Ortyn) in view of Nicoletti and Vitale.

4 Claims 42 and 43 have been canceled.

5 Novelty of Claim 1

6 Claim 1 recites a method for cell classification, using only spatial frequency data of a side scatter  
7 image of the cell. Spatial frequency data of a darkfield image of a known cell type is provided, then  
8 spatial frequency content data of a darkfield image of an unknown cell type is collected. If there is a  
9 match between the known spatial frequency data and the unknown spatial frequency data, positive  
10 identification of the cell type can be determined.

11 In the prior art, intensity data of side scattered light (SSC) from non-imaging cytometers was  
12 known to provide some information about the texture or internal complexity of a cell, while intensity data  
13 of forward scattered light (FSC) from non-imaging cytometers was known to provide some information  
14 about the relative size of a cell (Vitale, page 4, Background Information). However, these measurements  
15 were qualitative in nature, and were generally insufficient for conclusive classification of a cell. Cells  
16 having very different sizes and textures could exhibit similar intensity readings (for example, the intensity  
17 of a small bright cell could be the same as the intensity of a large dim cell), thus these measurements  
18 alone were generally not suitable for conclusive cellular identification.

19 The spatial frequency content of a side scattered image, the metric recited in Claim 1, is not  
20 equivalent to an intensity measurement from side scattered light. Both metrics use side scattered light, but  
21 the spatial frequency content is a much more complex metric than intensity. An inaccurate but useful  
22 analogy would be to consider a curve having a peak. The SSC intensity measurement is akin to the  
23 amplitude of the peak of the curve. An integration of the curve can be used to define the area under the  
24 curve, a much different and more complicated metric (the spatial frequency metric being akin to the  
25 integral of the curve, in that the spatial frequency metric provides a different, more complicated metric, as  
26 the integration of a curve does in comparison to the magnitude of the peak of a curve). The area under a  
27 curve and the amplitude of the peak of the curve are both metrics derived from the same curve, but they  
28 simply are not equivalent metrics. Similarly, SSC intensity measurements of a cell from a non-imaging  
29 cytometer are not equivalent to spatial frequency content metric of a SSC/darkfield image of the cell.

1 After developing an imaging cytometer, applicants began to study and manipulate the data (such  
2 as spatial frequency content of the side scatter/darkfield images, spatial frequency content of forward  
3 scatter/brightfield images, and spatial frequency content of fluorescent images) provided by the imaging  
4 cytometer they developed. They discovered that the *spatial frequency content of a side scatter image of a*  
5 *cell* provided sufficient information to conclusively identify a cell, so long as they had a reference image  
6 of a known cell so the *spatial frequency content of a side scatter image of an unidentified cell* could be  
7 matched to the *spatial frequency content of a side scatter image of a known cell*.

8 The cited art simply does not teach or suggest that biological cells can be conclusively identified  
9 by using the *spatial frequency content of a side scatter image of the cell* without using other metrics.

#### 10 Novelty of Claim 8

11 Claim 8 recites a method for cell classification, using only spatial frequency data of a brightfield  
12 image of the cell. Spatial frequency data of a brightfield image of a known cell type is provided, then  
13 spatial frequency content data of a brightfield image of an unknown cell type is collected. If there is a  
14 match between the known spatial frequency data and the unknown spatial frequency data, positive  
15 identification of the cell type can be determined.

16 The spatial frequency content of a brightfield image is not equivalent to an intensity measurement  
17 from forward scattered light. Both metrics use brightfield light, but the spatial frequency content is a  
18 much more complex metric than intensity.

19 The cited art recognized that the intensity of FSC light provides some information about the size  
20 of a cell. The cited art simply does not teach or suggest that the *spatial frequency content* of a brightfield  
21 image of a cell (an entirely different metric) provides sufficient information to conclusively identify a cell.

22 After developing an imaging cytometer, applicants began to study and manipulate the data (such  
23 as spatial frequency content of the side scatter/darkfield images, spatial frequency content of forward  
24 scatter/brightfield images, and spatial frequency content of fluorescent images) provided by the imaging  
25 cytometer they developed. They discovered that the *spatial frequency content of a brightfield image of a*  
26 *cell* provided sufficient information to conclusively identify a cell, so long they had a reference image of a  
27 known cell so the *spatial frequency content of a brightfield image of an unidentified cell* could be  
28 matched to the *spatial frequency content of a brightfield image of a known cell*.

29 The cited art simply does not teach or suggest that biological cells can be conclusively identified  
30 by using the *spatial frequency content of a brightfield image of the cell* without using other metrics.

1 Novelty of Claim 16

2 Claim 16 recites a method for cell classification, using only spatial frequency data of an image of  
3 the cell where the cell is stained with a single nuclear marker. Spatial frequency data of a known cell  
4 stained with that single nuclear marker is provided, then an image and spatial frequency content data of  
5 the stained unknown cell is collected. If there is a match between the known spatial frequency data and  
6 the unknown spatial frequency data, positive identification of the cell type can be determined.

7 As discussed above, the cited art does not teach or suggest using the spatial frequency content of  
8 an image of the cell to be used as a metric for classifying the cell, where the cell is stained only with a  
9 single nuclear dye.

10 Novelty of Claim 23

11 Claim 23 as amended specifically recites that by using a single nuclear stain and the spatial  
12 frequency content metric, one can determine which one of the following cell types the specific cell  
13 corresponds to:

14 *a viable cell having a cellular membrane that is impermeable to the nuclear marker;*  
15 *a cell in an early stage of apoptosis and which has a cellular membrane that is*  
16 *impermeable to the nuclear marker;*  
17 *a cell in a late stage of apoptosis and which has a cellular membrane that is*  
18 *permeable to the nuclear marker; and*  
19 *a necrotic cell which has a cellular membrane that is permeable to the nuclear*  
20 *marker.*

21 Vitale and Nicoletti can use stains, FSC intensity data, and SSC intensity data to classify cells as  
22 in one or more of those groups, ***but not all of those groups by using a single nuclear stain.***

23 Claim 23 recites a method for cell classification, using only spatial frequency data of an image of  
24 the cell where the cell is stained with a *single nuclear marker*. Spatial frequency data of a known cell  
25 stained with that single nuclear marker is provided, then an image and spatial frequency content data of  
26 the stained unknown cell is collected. If there is a match between the known spatial frequency data and  
27 the unknown spatial frequency data, positive identification of the cell type can be determined.

28 As discussed above, the cited art does not teach or suggest using the ***spatial frequency content of***  
29 ***an image of the cell*** to be used as a metric for classifying the cell. Further, none of the cell classifications  
30

1 techniques in the cited art can identify a cell as being one of the four specified types using only a single  
2 stain/nuclear dye.

3 Nicoletti discloses a first technique (see pages 3-6) that uses a single nuclear stain to distinguish  
4 between late state apoptotic cells, necrotic cells, and viable cells, but that technique cannot distinguish  
5 between viable cells and early stage apoptotic cells where the cellular membrane is still intact. Thus, that  
6 is not an equivalent technique, because it does not employ the *spatial frequency content of an image of*  
7 *the cell* nor can that technique determine if the cell is an early apoptotic cell.

8 Nicoletti discloses a DNA break staining technique that can separate early apoptotic cells (having  
9 more DNA breaks than other cell types, thus a relatively high fluorescent signal from the break dye is  
10 indicative of early apoptotic cells) from necrotic cells and viable cells, but that technique does not  
11 distinguish between the viable and necrotic cells (both have low stain signals). Thus, that is not an  
12 equivalent technique, because it does not employ the *spatial frequency content of an image of the cell*  
13 nor can that technique determine if the cell is viable versus necrotic (both have low stain signals).

14 Nicoletti discloses an Annexin-V stain that enables early apoptotic cells to be distinguished from  
15 viable or necrotic cells, but that technique cannot determine whether a specific cell that is negative for  
16 Annexin-V result is viable or necrotic. Thus, that is not an equivalent technique, because it does not  
17 employ the *spatial frequency content of an image of the cell* nor can that technique conclusively  
18 determine which one of the four defined cell types the specific cell actually is.

19 Vitale discloses using a combination of FSC intensity measurements and SSC intensity  
20 measurements to classify cells as necrotic, late apoptotic, and viable. Note this technique cannot identify  
21 early apoptotic cells. Thus, that is not an equivalent technique, because it does not employ the *spatial*  
22 *frequency content of an image of the cell* nor can that technique determine if the cell is an early apoptotic  
23 cell.

24 Vitale discloses a nuclear staining technique (the SSC and FSC technique above is not based on  
25 staining) which employs *two* nuclear stains to facilitate the classification of cells as necrotic or apoptotic  
26 (bottom of page 7). Apoptotic cells show high HO staining and low PI staining, necrotic cells are brightly  
27 stained by PI, and healthy cells are dimly stained by HO and not stained by PI. That is not an equivalent  
28 technique, because it does not employ the *spatial frequency content of an image of the cell* nor does this  
29 technique use only a single stain. Furthermore, because cells in the early stage of apoptosis have intact  
30

1 cell membranes, they respond to nuclear stains like viable cells, and it does not appear that this technique  
2 can determine if the cell is an early apoptotic cell.

3 The modifications required to enable only a single nuclear stain to be used in connection with  
4 spatial frequency content data of an image of the cell to determine which of the four types the cell should  
5 be classified as appear to be significant, and non-obvious.

#### 6 Novelty of Claim 29

7 Claim 29 as amended specifically recites that by using a single nuclear stain and the spatial  
8 frequency content of a side scattered image of the cell, one can determine which one of the following cell  
9 types the specific cell corresponds to: a viable cell, a necrotic cell, a late apoptotic cell, or an early  
10 apoptotic cell. As discussed above in connection with the novelty of Claim 23, the cited art does not  
11 teach or suggest using the spatial frequency metric for a cell classifier for the four identified cell types, or  
12 any technique that can determine which of the four cells types is present using only a single nuclear stain.

#### 13 Novelty of New Claims 45, 46, 47, and 48

14 New Claims 45-48 recite a definition of the term spatial frequency content, based on the  
15 specification as filed at paragraph [0045]. While applicants believe that the term *spatial frequency* is  
16 understood in the art, and that the plain meaning of the term as understood in the image processing arts  
17 should be used in examining the claims (per MPEP 2111.01), the specification does clearly disclose that  
18 the spatial frequency metric can be calculated by *computing the standard deviation of the individual*  
19 *pixel intensities* within the image. While that portion of the text is specifically referring to a darkfield  
20 image, the metric (*the standard deviation of the individual pixel intensities*) can be determined for  
21 any image (brightfield, darkfield, fluorescent, etc.). Applicants believe they are the first to recognize  
22 the utility of that metric to the cell classifications techniques disclosed herein.

#### 23 Amendments to Claims 30, 31, 33, 34, 36, 37, 39 and 40

24 The amendments to the these claims are based on FIGURE 5 and the corresponding text.

25 FIGURE 5 is a fairly complex Figure which includes quite a lot of detail. The central portion of  
26 FIGURE 5 is a scatter plot of a complex morphological feature determined by analyzing multispectral  
27 image data acquired from many different cells. FIGURE 5 also includes four panels of images of  
28 exemplary cells from each of the following types: necrotic cells, cells in an early stage of apoptosis,  
29 cells in a late stage of apoptosis, and viable cells. Significantly, a complex morphological feature  
30 derived in part from the spatial frequency data acquired from a multispectral imaging system using

1 cells only stained with a single nuclear stain enabled a population of cells to be sorted into necrotic  
2 cells, cells in an early stage of apoptosis, cells in a late stage of apoptosis, and viable cells. While the  
3 prior art has provided techniques for distinguishing between some of those cell types, the prior art has  
4 not provided a technique which *enables all four of those cell types to be distinguished from one*  
5 *another using a single analysis and a single nuclear stain* (other techniques either require multiple  
6 stains, or multiple analytical techniques).

7 As discussed in the specification, the presence of the nuclear stain in the nucleus means the cell  
8 must be late apoptotic or necrotic (viable and early apoptotic cells have intact membranes that keep the  
9 stain out of the nucleus). Once the presence/absence of the stain separates the cells into two groups, the  
10 spatial frequency metric can be used to further classify the cells. Cells with no stain are viable or early  
11 apoptotic, and if the spatial frequency metric matches known viable cells, the specific cell is viable, while  
12 if the spatial frequency metric does not match known viable cells, the specific cell is early apoptotic.  
13 Similarly, if the specific cell is not stained, and if the spatial frequency metric matches known early  
14 apoptotic cells, the specific cell is early apoptotic, while if the spatial frequency metric does not match  
15 known early apoptotic cells, the specific cell is viable.

16 Cells with stain are necrotic or late apoptotic, and if the spatial frequency metric matches known  
17 necrotic cells, the specific cell is necrotic, while if the spatial frequency metric does not match known  
18 necrotic cells, the specific cell is late apoptotic. Similarly, if the specific cell is stained, and if the spatial  
19 frequency metric matches known late apoptotic cells, the specific cell is late apoptotic, while if the spatial  
20 frequency metric does not match known late apoptotic cells, the specific cell is necrotic.

21 In consideration of the amendment to the claims and the Remarks set forth above, it is applicants'  
22 position that all claims in the current application are patentable over the art of record. The Examiner is  
23 thus requested to pass this case to issue without further delay. In the event that any other issues remain,  
24 the Examiner is invited to telephone applicants' attorney at the number listed below.

25 Respectfully submitted,

26  
27 /mike king/  
28 Michael C. King  
29 Registration No. 44,832

30 MCK/RMA:elm